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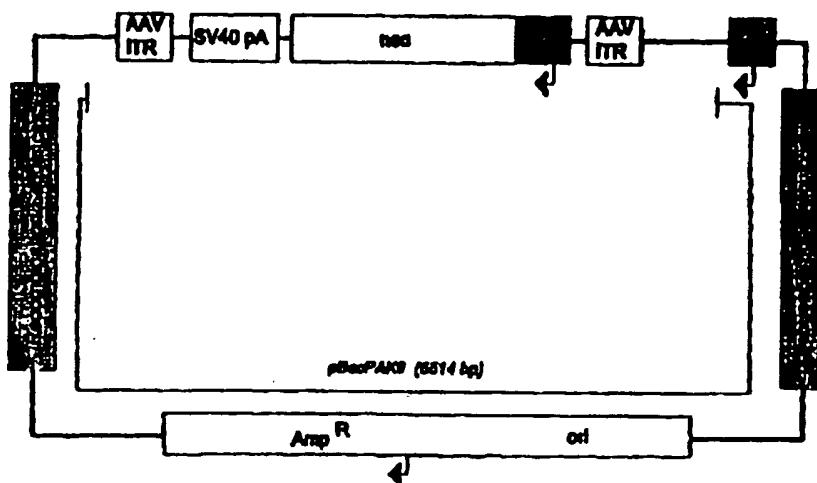


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(54) Title: USE OF A NON-MAMMALIAN DNA VIRUS TO EXPRESS AN EXOGENOUS GENE IN A MAMMALIAN CELL

**AcMNPV Transfer Plasmid pBV-AVneo**



(57) Abstract

Disclosed is a method of expressing an exogenous gene in a mammalian cell, involving infecting the cell with a non-mammalian virus, such as a baculovirus, whose genome carries an exogenous gene, and growing the cell under conditions such that the gene is expressed. Exogenous genes are delivered to mammalian cells by use of a transfer vector such as that described in the figure. Also disclosed is a method of treating a gene deficiency disorder in a mammal by providing to a cell a therapeutically effective amount of a virus whose genome carries an exogenous gene and growing the cell under conditions such that the exogenous gene is expressed in the mammal.

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## AMENDED CLAIMS

[received by the International Bureau on 25 March 1996 (25.03.96);  
original claims 18 and 58 amended; new claims 63-103 added;  
remaining claims unchanged (9 pages)]

10. The method of claim 5, wherein said  
baculovirus is in the budded form.

11. The method of claim 1, wherein said genome  
further comprises a promoter of a long-terminal repeat of  
5 a transposable element.

12. The method of claim 1, wherein said genome  
further comprises a promoter of a long-terminal repeat of  
a retrovirus.

13. The method of claim 12, wherein said  
10 retrovirus is Rous Sarcoma Virus.

14. The method of claim 1, wherein said genome  
further comprises an integrative terminal repeat of an  
adeno-associated virus.

15. The method of claim 14, wherein said genome  
15 further comprises an adeno-associated virus rep gene.

16. The method of claim 1, wherein said genome  
further comprises a cell-immortalizing sequence.

17. The method of claim 1, wherein said genome  
further comprises an origin of replication.

20 18. The method of claim 17, wherein said origin  
of replication comprises an Epstein Barr virus origin of  
replication.

19. The method of claim 1, wherein said genome  
further comprises a polyadenylation signal and an RNA  
25 splicing signal.

medicament for treating a gene deficiency disorder in a mammal.

55. The use of claim 54, wherein said virus is an invertebrate virus.

5 56. The use of claim 55, wherein said invertebrate virus is an insect virus.

57. The use of claim 56, wherein said insect virus is a baculovirus.

58. The use of claim 54, wherein said gene  
10 encodes a gene product selected from the group consisting of fumarylacetoacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, low-density-lipoprotein receptor, porphobilinogen deaminase, carbamoyl synthetase I, ornithine  
15 transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, factor VIII, factor IX, cystathione  $\beta$ -synthase, branched chain ketocacid decarboxylase, albumin, isovaleryl-CoA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase,  
20 glutaryl CoA dehydrogenase, insulin,  $\beta$ -glucosidase, and pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, Menkes disease protein, the product of Wilson's disease gene PWD and CFTR.

25 59. Use of a non-mammalian DNA virus whose genome comprises a carcinoma-therapeutic gene selected from the group consisting of tumor necrosis factor, thymidine kinase, diphtheria toxin chimeras, and cytosine deaminase in the preparation of a medicament for treating  
30 hepatocellular carcinoma in a mammal.

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60. The use of claim 59, wherein said non-mammalian DNA virus is a baculovirus.

5 61. The use of claim 59, wherein said carcinoma-therapeutic gene is operably linked to an  $\alpha$ -fetoprotein promoter.

10 62. The method of claim 1, wherein said exogenous gene is selected from the group consisting of lacZ genes, chloramphenicol acetyltransferase genes, alkaline phosphatase genes, luciferase genes, and green fluorescent protein genes.

15 63. A nucleic acid comprising:  
a genome of a non-mammalian DNA virus;  
an exogenous mammalian gene; and  
an exogenous mammalian-active promoter, wherein said gene is operably linked to said promoter.

20 64. The nucleic acid of claim 63, wherein said genome is the genome of an insect virus.

65. The nucleic acid of claim 64, wherein said genome is the genome of a baculovirus.

25 66. The nucleic acid of claim 65, wherein said genome is the genome of an *Autographa californica* multiple nuclear polyhedrosis virus.

30 67. The nucleic acid of claim 63, wherein said mammalian-active promoter is selected from the group consisting of mammalian promoters, promoters of long-terminal repeats of retroviruses, promoters of long-terminal repeats of transposable

elements, the Simian Virus 40 early promoter, the cytomegalovirus IE promoter, and the adenovirus major late promoter.

5 68. The nucleic acid of claim 67, wherein said promoter is a mammalian promoter.

69. The nucleic acid of claim 63, wherein said promoter is selected from the group consisting of cell-type-specific promoters, stage-specific promoters, inducible promoters and  
10 tissue-specific promoters.

70. The nucleic acid of claim 69, wherein said promoter is a liver-specific promoter.

15 71. The nucleic acid of claim 70, wherein said liver-specific promoter is selected from the group consisting of hepatitis B promoters, hepatitis A promoters, hepatitis C promoters, albumin promoters,  $\alpha$ -1-antitrypsin promoters, pyruvate kinase promoters, phosphoenol pyruvate carboxykinase promoters, transferrin promoters, transthyretin promoters,  $\alpha$ -fetoprotein  
20 promoters,  $\alpha$ -fibrinogen promoters, and  $\beta$ -fibrinogen promoters.

72. The nucleic acid of claim 70, wherein said liver-specific promoter is selected from the group consisting of low  
25 density lipoprotein receptor promoters,  $\alpha$ 2-macroglobulin promoters,  $\alpha$ 1-antichymotrypsin promoters,  $\alpha$ 2-HS glycoprotein promoters, haptoglobin promoters, ceruloplasmin promoters, plasminogen promoters, complement protein promoters, C3 complement activator promoters,  $\beta$ -lipoprotein promoters, and  $\alpha$ 1-  
30 acid glycoprotein promoters.

73. The nucleic acid of claim 63, further comprising a mammalian origin of replication.

74. The nucleic acid of claim 63, further comprising an integrative terminal repeat.

5 75. The nucleic acid of claim 63, wherein said genome lacks a functional polyhedron gene.

76. The nucleic acid of claim 63, wherein said gene is a human gene.

10 77. The nucleic acid of claim 63, wherein said gene is a therapeutic gene.

78. The nucleic acid of claim 63, wherein said gene encodes a gene product selected from the group consisting of carbamoyl  
15 synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase fumarylacetoacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, low-density-lipoprotein receptor, porphobilinogen deaminase, arginase, factor VIII, factor IX,  
20 cystathione  $\beta$ -synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-CoA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin,  $\beta$ -glucosidase, and pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-  
25 protein, Menkes disease protein, the product of Wilson's disease gene pWD, growth factors, interferons, CFTR, tumor suppressors, herpes simplex virus thymidine kinase, and transcription factors.

79. A nucleic acid comprising:  
30 a genome of a non-mammalian DNA virus;  
an exogenous antisense RNA gene, the RNA encoded by said gene being complementary to a nucleic acid of a gene that is expressed in a cell at an undesirably high level; and

an exogenous mammalian-active promoter, wherein said gene is operably linked to said promoter.

5 80. The nucleic acid of claim 79, wherein said genome is the genome of an insect virus.

81. The nucleic acid of claim 79, wherein said genome is the genome of a baculovirus.

10 82. The nucleic acid of claim 79, wherein said promoter is selected from the group consisting of mammalian promoters, promoters of long-terminal repeats of retroviruses, and promoters of long-terminal repeats of transposable elements, the Simian Virus 40 early promoter, the cytomegalovirus IE promoter, and the  
15 adenovirus major late promoter.

83. A cell that contains a nucleic acid, wherein said nucleic acid comprises:

20 a genome of a non-mammalian DNA virus;  
an exogenous mammalian gene; and  
an exogenous mammalian-active promoter, wherein said gene is operably linked to said promoter.

25 84. The cell of claim 83, wherein said genome is the genome of an insect virus.

85. The cell of claim 85, wherein said genome is the genome of a baculovirus.

30 86. The cell of claim 83, wherein said promoter is selected from the group consisting of mammalian promoters, promoters of long-terminal repeats of retroviruses, and promoters of long-terminal repeats of transposable elements, the Simian Virus 40



early promoter, the cytomegalovirus IE promoter, and the adenovirus major late promoter.

5 87. The cell of claim 83, wherein said cell is a primary cell.

88. The cell of claim 83, wherein said cell is a human cell.

10 89. The cell of claim 83, wherein said cell is selected from the group consisting of hepatocytes, kidney cells, NIH3T3 cells, HeLa cells, Cos7 cells, C<sub>2</sub>C<sub>12</sub> myotubes, C<sub>2</sub>C<sub>12</sub> myoblasts, CHO/dhfr<sup>-</sup> cells, lung cells, and PC12 cells.

15 90. The cell of claim 89, wherein said cell is a hepatocyte selected from the group consisting of HepG2 cells, Sk-Hep-1 cells, Hep3B cells, FTO2B cells, and Hepa 1-6 cells.

20 91. The cell of claim 83, wherein said cell is selected from the group consisting of Ramos cells, Jurkat cells, HL60 cells, and K-562 cells.

25 92. The cell of claim 83, wherein said promoter is selected from the group consisting of cell-type-specific promoters, tissue-specific promoters, stage-specific promoters, and inducible promoters.

30 93. The cell of claim 83, wherein said promoter is a liver-specific promoter.

94. The cell of claim 83, wherein said gene is a human gene.

95. The cell of claim 83, wherein said gene encodes a gene product selected from the group consisting of carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase fumarylacetoacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, low-density-lipoprotein receptor, porphobilinogen deaminase, arginase, factor VIII, factor IX, cystathione  $\beta$ -synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-CoA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin,  $\beta$ -glucosidase, and pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, Menkes disease protein, the product of Wilson's disease gene pWD, growth factors, interferons, CFTR, tumor suppressors, herpes simplex virus thymidine kinase, and transcription factors.

96. A nucleic acid comprising:  
a genome of a non-mammalian DNA virus;  
an exogenous cancer therapeutic gene selected from the group consisting of tumor necrosis factor genes, thymidine kinase genes, chimeric diphtheria toxin genes, and cytosine deaminase genes; and  
an exogenous mammalian-active promoter, wherein said gene is operably linked to said promoter.

25

97. The nucleic acid of claim 96, wherein said genome is the genome of an insect virus.

98. The nucleic acid of claim 97, wherein said genome is the genome of a baculovirus.

30

99. A nucleic acid comprising:  
a genome of a non-mammalian DNA virus;

an exogenous gene selected from the group consisting of RNA  
decoy genes and ribozyme genes; and  
an exogenous mammalian-active promoter.

5           100. The nucleic acid of claim 99, wherein said genome  
comprises the genome of a baculovirus.

101. A pharmaceutical composition comprising:  
(A) a pharmaceutically acceptable excipient and  
10       (B) a nucleic acid, said nucleic acid comprising:  
          a genome of a non-mammalian DNA virus;  
          an exogenous mammalian gene; and  
          an exogenous mammalian-active promoter, wherein said  
gene is operably linked to said promoter.

15           102. The pharmaceutical composition of claim 101, wherein  
said genome comprises the genome of an insect virus.

20           103. The pharmaceutical composition of claim 102, wherein  
said genome comprises the genome of a baculovirus.